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A unifying concept for the dependence of whole crop N:P ratio on biomass: theory and experiment

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Running Title: **N:P ratio and plant biomass**

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Abstract

- *Background and aims* Numerous estimates have been made of the concentrations of N and P required for good growth of crop species but they have not been defined by any unifying model. Our aim was to develop such a model for the dependence of N:P ratio on crop mass, to test its validity and to use it to identify elements of similarity between different crop species and wild plants.
- *Methods* A model was derived between plant N:P ratio (R_w) and its dry biomass per unit area (W) during growth with near optimum nutrition by considering that plants consist of growth related tissue and storage related tissue with N:P ratios R_g and R_s respectively. Testing and calibration against experimental data on different crop species led to a simple equation between R_w and W which was tested against independent experimental data.
- *Key results* The validity of the model and equation was supported by 365 measurements of R_w in 38 field experiments on crops. R_g and R_s remained approximately constant throughout growth, with average values of 11.8 and 5.8 by mass. The model also approximately predicted the relationships between leaf N and P concentrations in 124 advisory estimates on immature tissues and in 385 wild species from published global surveys.
- *Conclusions* The N:P ratio of the biomass of very different crops, during growth with near optimum levels of nutrients, is defined entirely in terms of crop biomass, an average N:P ratio of the storage/structure related tissue of the crop and an average N:P ratio of the growth related tissue. The latter is similar to that found in leaves of many wild plant species and even microorganisms and terrestrial and fresh water autotrophs.

Key words: Biomass, growth rate, model, N:P ratio, plant allometry, plant mass, stoichiometry.

INTRODUCTION

Elucidating changes in N:P ratios during plant growth and developing mechanistic models for them could help solve major problems in crop, environmental and ecological sciences (Sterner and Elser, 2002; Sadras, 2006). For example in the context of crop production, although numerous measurements have been made on the N and P concentrations in leaves as an aid to diagnosing deficiencies it is not clear how far the differences in estimated "optimum" concentrations result from experimental error, differences in plant age, plant mass, leaf position or differences between species. In addition uncertainty about the changes in the N:P ratio of crop biomass during growth has proved to be a serious problem in simulating the interactive effects of N and P on crop growth (Zhang *et al*., 2007). A model with a strong theoretical background is needed to explain some of the uncertainties in the estimated optimum nutrient concentrations and thus contribute to a more effective use of added nutrients. Such a model might also be useful in detecting atmospheric N-deposition damage to natural vegetation (Güsewell, 2005), and in establishing the effects of imbalance of nutrients on natural vegetation (Güsewell, 2004).

The possibility that such a model could be devised is suggested by the discovery of important principles and relationships that emphasise the similarities in the N and P dynamics in different organisms. The specific growth rates (SGR) of heterotrophs are generally strongly and positively related to their contents of RNA and thus to their P:N ratios (Sterner and Elser, 2002). The Growth Rate Hypothesis was proposed to encompass this relationship (Elser *et al*., 1996, Elser *et al*., 2000b, Sterner and Elser 2002) and it applies not only across different microorganisms, but also at the level of the individual species (Karpinets *et al*., 2006). Ågren (2004) has further developed the theory for algae and tree seedlings. On the basis of

biochemical considerations he predicts that their SGR may increase or decrease with increase in P:N ratio depending on whether SGR is high or low. Measurements on leaves of wild herbaceous species backed up by theory indicate that SGR increased with increase in P:N ratio (Niklas, 2006). Strong correlations were also found at the species level between shoot concentrations of P and N in 117 angiosperm species grown in hydroponic culture with a constant nutrient regime (Broadley *et al*., 2004).

Lack of knowledge about the changes in N:P ratios during the growth of the entire biomass of both crop and of natural vegetation is particularly conspicuous. Thus in one survey of natural vegetation Kerkhoff and Enquist (2006) found that the N:P ratios were approximately the same, about 11, over a wide range of plant weights whereas in another study it was found that the N:P ratios were greater in the leaves than in the stems and roots of herbage crops which implies that the N:P ratio decreased with increase in plant mass (Kerkhoff *et al*., 2006). Agricultural studies are sparse but a recent review (Sadras, 2006) indicates that N:P ratios of cereals varied between 1 and 20 . A major cause of the variability lies in variations in the supply of nutrients to crops and, in particular, the tendency of crops to absorb far more P than is needed to meet the immediate needs and to store it (Bollons and Barraclough, 1999). However this cannot explain the variability in the N:P ratio when there is an optimal supply of nutrients. Our work was therefore initially focussed on crop biomass N:P ratio during growth of very different crop species receiving near optimum levels of nutrients.

Models considering plants to consist of at least two types of tissues have been developed for the decline of N concentration of the whole plant with increasing plant mass (Caloin and Yu, 1982; Caloin and Yu, 1984; Hardwick, 1987; Lemaire and Gastal, 1997). Caloin and Yu (1984) developed a model that predicted a linear increase of plant N concentration with rate SGR. The prediction was confirmed by a controlled environment experiment and later by field experiments

of others (Greenwood *et al*., 1991). These studies suggest that an approach based on the relative rates of growth of different plant tissues could lead to a unifying mechanistic insight into relationships between N:P concentrations and plant biomass.

The objective of our work was to develop a mechanistic model along these lines for the changes in N:P ratio of whole crop biomass during growth with near optimum nutrition and to test its validity against a wide range of experimental data. We established that processes governing N:P ratios are defined by the same equations for different species. On this basis we have developed a model for the changes in N:P ratio with increase in crop mass. We have tested its validity and calibrated it with experimental data obtained with near optimum nutrients. This led to a simple equation which we tested against data that was entirely independent of that used for developing the model. It enables predictions to be made about the N:P ratios of plant biomass and of leaves. We believe that the proposed model and the equation and associated principles could be useful in advancing the crop, environmental and ecological sciences.

MATERIALS AND METHODS

Model development

Definitions of the symbols and the units are given in the Appendix Table A1. The model is concerned with changes in the N:P ratio of whole crop dry weights, excluding fibrous roots, that occur during growth with optimum levels of nutrients before the onset of senescence. We consider that most of the changes arise because of a decline in the tissues related to growth relative to those that are related to storage and structure. More specifically we consider that

growth related tissues predominate in leaves and the storage and structure related tissues, which will be referred as the storage related tissue, in the remaining parts of the plant. According to this classification, Kerkhoff *et al*., (2006) found that in natural herbage the N:P ratio of the storage related tissue is lower than in the growth related tissue. Thus, as plants get larger the proportion of storage related tissue to growth related tissue would be expected to increase and the N:P ratio to decline. We propose that at the beginning of growth, plants are mainly comprised of the proliferating cells organized in metabolically active, mainly growth plant tissues, which will be further referred as growth related tissue (W_g). The N:P ratio of the plant organism at this stage is considered to be similar to those of exponentially growing unicellular organisms. As the plant gets bigger the proportion of W_g in the plant biomass decreases with a concomitant decrease in *SGR*; at the same time there is an increase in the proportion of storage related tissue (W_s). On this basis we derive a simple relationship between N:P ratio of the whole crop (R_w) and crop dry biomass per unit area (W) with two constants that represent an average N:P ratio in the growth related tissue (R_g) and an average N:P ratio in the storage related tissue (R_s). The relationship is based on the following three assumptions.

A. The growth rate of the crop is always proportional to the weight of growth related tissue (W_g)

$$dW/dt=\alpha \cdot W_g \qquad (1)$$

where W is in Mg ha^{-1} and t is in days and α is a proportionality constant.

B. Growth rate with the optimum levels of nutrients is defined by

$$dW/dt = K_2 \cdot W/(K_1 + W) \quad (2)$$

where K_2 (Mg ha^{-1} d^{-1}) is the growth rate coefficient and K_1 (Mg ha^{-1}) is a constant. The last coefficient represents the value of W when growth rate is half the maximum. The equation was derived by considering that interception of radiation by the crop and thus dW/dt increases asymptotically with W until the onset of senescence (defined as when growth rate starts to decline) and it has been validated by experiments on field vegetables during the main UK growing season (Greenwood *et al*., 1977).

By considering the previous assumption (assumption A) dW/dt can be eliminated from eqns (1) and (2), which gives

$$\alpha \cdot W_g = K_2 \cdot W/(K_1 + W) \quad (3)$$

C. At the beginning of growth the crop biomass (W) is comprised only of growth related tissues, i.e. as $W \rightarrow 0$, $W = W_g$

This assumption about the initial growth condition is important as it permits the estimation of the coefficient "α" in the eqn (3). Indeed, if crop biomass is small, i.e. $W \rightarrow 0$, $K_2 \cdot W/(K_1 + W)$ can be replaced with $W \cdot K_2/K_1$. But according to the assumption C, crop biomass at the beginning of growth is comprised only of growth related tissue or $W = W_g$. Therefore $\alpha \cdot W_g = W_g \cdot K_2/K_1$ or $\alpha = K_2/K_1$. As α remains constant throughout growth (assumption **A**), $K_2/K_1 = \alpha$ may be substituted in eqn (3) to give

$$W_g = K_1 \cdot W/(K_1 + W) \quad (4)$$

and

$$W_s = W - K_1 \cdot W/(K_1 + W) \quad (5)$$

Let P_g and P_s be the total weights of P and N_g and N_s the total weights of N in the growth and the storage related tissues respectively, then the ratio of N:P in the whole plant (R_w) is given by

$$R_w \equiv\equiv (N_g + N_s)/(P_g + P_s) \quad (6)$$

Multiplying throughout by (P_g+P_s) and then dividing by P_g gives

$$R_w + R_w P_s/P_g = N_g/P_g + N_s P_s/P_s P_g$$

which on rearranging and substituting the N:P ratios of the growth and storage related tissues respectively, namely $R_g = N_g/P_g$ and $R_s = N_s/P_s$ gives

$$R_w = [R_g + R_s(P_s/P_g)]/(1 + P_s/P_g) \quad (7)$$

Let C_{Pg} and C_{Ps} be the concentrations of P in the growth and storage related tissues then $P_s = W_s \cdot C_{Ps}$ and $P_g = W_g \cdot C_{Pg}$ which when substituted in eqn (7) gives

$$R_w = \left[R_g + R_s\left(\frac{W_s C_{Ps}}{W_g C_{Pg}}\right)\right] / \left[1 + \left(\frac{W_s C_{Ps}}{W_g C_{Pg}}\right)\right]$$

Measured differences in P concentration between different plant organs usually differ by a factor of less than 2 (e.g. Ingestad and Lund, 1979; Greenwood *et al*., 1980b; de Groot *et al*., 2003; Kerkhoff *et al*., 2006). The effect of varying C_{Ps}/C_{Pg} on R_W was calculated with W varying from 0.1 to 20 Mg ha^{-1}, with W_s and W_g calculated from W by eqns (4) and (5) and with R_g and R_s having the average values found for the different species of field vegetables. This sensitivity analysis given in **[Table S1 in Supplementary Information]** indicated that R_w was almost unaffected by C_{Pg}/C_{Ps} when W was either small or large. And even over the entire range, a change in C_{Pg}/C_{Ps} by ±50% on average only resulted in a less than 10% change in R_w. Thus although C_{Pg}/C_{Ps} deviates considerably from unity, setting C_{Pg}/C_{Ps} = 1 will result in little error. We therefore consider that W_g/W_s can be taken as equal to P_g/P_s which when substituted in eqn (7) gives

$$R_w = [R_g + R_s(W_s/W_g)]/[(W_g+W_s)/W_g] \quad (8)$$

and as $W = W_g + W_s$, eqn (8) can rewritten as

$$R_w = [R_g \cdot W_g + R_s \cdot W_s]/W \quad (9)$$

Substitution in this equation of W_g and W_s by eqns (4) and (5) gives

$$R_w = [(K_1(R_g-R_s)/(K_1+W)] + R_s \quad (10)$$

The last equation is a linear relationship between the crop N:P ratio and the dry biomass related function $K_1/(K_1+W)$ with two constants (R_g-R_s) and R_s.

Datasets

Details of all three datasets are given in **[Table S2 in Supplementary Information].**

Dataset A. Most measurements in the dataset are from 24 experiments carried out at Wellesbourne, UK. In these experiments 14 different species of field vegetables were grown in the same field with near optimum levels of N, P and K fertilizers (Greenwood *et al*., 1977; Greenwood *et al*., 1980a). In each experiment there were between 5 and 21 harvests with measurements of W, and of N and P contents, made at intervals during the growing period until the commercial harvest date which is before the onset of senescence. The near optimum levels of fertilizer had, depending on the crop, N:P ratios of from 0.7 to 5.4 and gave average yields that were 92% of the maximum achieved with any combination of levels of fertilizer-nutrients in an adjacent multilevel NPK fertilizer experiment on the same crop. The remaining experiment in this dataset was at Lerida, NE Spain (Bosch Serra, 1999) on a Xerofluvent aquic, fine silty, mixed (calcareous) messic soil (Soil Survey Staff, 1975). Onions were drilled in March, and at 50% emergence were thinned to 80 plants m^{-2}. They were drip irrigated to maintain the water potential higher than -18kPa and were fertigated at 334 kg N ha^{-1}, 62 kg P ha^{-1}, and 403 kg K ha^{-1}. Pest disease and weed control were effective.

Altogether there were 287 measurements on 15 different species (Table 1). A special feature of these experiments is that there is a sufficient proportion of measurements (43%) with small values of W (W< 2 t h^{-1}) to provide a sensitive test of the model.

Datasets A was also used to test the validity of the integrated form of eqn (2) namely

$$K_2 t = K_1 \cdot \ln W + W + C \qquad (11)$$

where C is the constant of integration and where W is crop dry weight (excluding fibrous roots in Mg ha^{-1} at time t in days and K_1 is equal to 1 Mg ha^{-1} for field vegetables as this had been found to give good fits to past field experimental data (Greenwood *et al*., 1977; Greenwood *et al*., 1985). K_2 is a growth rate coefficient which is generally constant throughout growth until senescence; in effect it corrects growth for differences in plant mass. $K_1 \ln W + W$ was regressed against t for each experiment to provide estimates of K_2; on average the regressions had an r^2 of 0.96 (Table 1).

Dataset B. This dataset was obtained by searching the literature for measurements at intervals, during growth with near optimal nutrition, of W, %N, and %P that were not included in dataset A. It consists of 78 sets of measurements from 13 experiments in 5 countries; there were 6 different crops of which 5 were not in dataset A. The data was also different from that in dataset A, because it contained few measurements where W was less that 2 Mg ha^{-1} and, thus, did not allow satisfactory estimates of R_g. It was used to test the predicted relationship between N:P ratio and W for W between 0.1 and 27 Mg ha^{-1}. It was also used to confirm the finding from dataset A that the time course dry crop weight increase followed eqn (11) **[Table S2 in Supplementary Information].**

The measured average N:P ratio is 7.9 in dataset A and 7.2 in dataset B compared with an average of 7.2 in the particulate matter in the sea which is the same as the average ratio in sea water (Sterner and Elser, 2002, p29).

Dataset C. Plant analyses are often used to guide fertilizer practice. Many experiments have been carried out to find the "optimum or adequate concentrations" at different stages of growth of many crop species. Tcierling (1978) deduced such concentrations from cereal experiments in

Russia and Bergman (1992) deduced them for numerous crop species from experiments in a wide range of countries. Dataset C includes measurements taken directly from the Tcierling's review (Tcierling 1978). Bergman (1992) gives the upper and lower values of the adequate range of %N and %P; the mean values of each of these two values are also given in dataset C and these were compared with the predictions as described below. Tcierling's data consisted of values of %N and %P (for the above ground parts of the plant) at different growth stages of cereals.

Neither the Tcierling nor the Bergman data included values of W, which is an essential input into eqn (10) after calibration to form eqn (15), as will be described in the Results section. Apparent values of W in the dataset were calculated from inputs of %N by

$$Optimum\ \%N = 1.35(1+\beta e^{-0.26W}) \qquad (12)$$

(Greeenwood and Draycott, 1989) with β =3 which had been found to be satisfactory for many field vegetables and has been used widely (Zhang et al., 2007). For the Tcierling data eqn (12) was used to produce a set of corresponding values of %N, and W. R_w was calculated by inserting these calibrated values of W into eqn (15). The Bergman (1992) data covered a considerable range of crop species and of %P and %N. These mean values for each species were compared with a predicted relationship between %P and %N. To generate the prediction we first used eqn. (12) to calculate %N for W = 2 t ha^{-1} to 20 t ha^{-1} in steps of 1 t ha^{-1}. The same values of W were then used to calculate R_w by inserting into eqn (15) and to predict %P multiplying $1/R_w$ by %N. These values of %P were regressed against those of %N to give a predicted linear relationship that was compared with Bergman's data in Fig. 5. The same procedure but with a different range of values of W was used to calculate a predicted relationship between %P and %N for

comparison with experimental data on grasses reported by (Salette, 1990) given in Fig.6. Thus, these predicted linear relationships were independent of the data with which they were compared.

Regression analysis. Whenever both variables were subject to error regressions were by the Reduced Major Axis (RMA) procedure (Niklas, 2006) with software by Bohonak (2004). If only one variable was subject to error, regression was by the standard procedure.

RESULTS

1. The N:P ratio of the whole plant declines with increase in plant mass during growth

The validity of eqn (10) was tested by regression of plant N:P ratio (*Rw*) against *1/(1+W)*, as K_1 = 1 Mg ha^{-1}, for the measurements in dataset A. Fig. 1 gives regressions for those experiments with most measurements and illustrates that the points were generally randomly scattered about the best fitting line; Table 1 gives values of r^2 averaged over the experiments for each crop. They show that measurements of *W* and N:P ratio during growth fit eqn (10) closely. Thus this theoretical equation gives good predictions for all crops of the linear increase in the ratio as a function of *1/(1+W)*. Therefore the ratio decreases with increase in *W*.

2. The N:P ratio of the whole plant declines with decrease in the plant specific growth rate during growth

Strong evidence that, for a given experiment, eqn (2) with the same value of K_2 held throughout growth is provided by the excellent fits (average r^2 for each experiment = 0.96) to eqn (11), the

integral of eqn (2) for dataset A (Table 1). Similar results were obtained for dataset B **[Table S2 in Supplementary Information]**. From eqn (2)

$$SGR = \frac{dW}{dt}\frac{1}{W} = K_2/(K_1+W) \qquad (13)$$

Substitution of K_2/SGR for (K_1+W) of this eqn in eqn (10) gives

$$R_w = [K_1(R_g-R_s).\ SGR/K_2] + R_s \qquad (14)$$

Assuming all of the parameters except R_w and SGR are constant as is indicated by Table 1 it follows that during a given experiment R_w decreases with decrease in SGR.

3. Average N:P ratios are similar among crops at the level of growth related tissue or of storage related tissue and do not change significantly during plant growth

According to eqn (10) the calculated regression coefficients (intercept and slope) allow estimates to be made of the N:P ratio in the growth related tissue (R_g) and in the storage related tissue (R_g) for each crop (Table 1). Because there were two independent experiments on most crops, an analysis of variance of the values of R_g and of R_s was carried out. The standard errors for the differences between crop means, R_g and R_s are given at the bottom of Table 1. Both R_g and R_s did not differ significantly between most crops. Given this similarity the average values of R_g and R_s, which are 11.83, and 5.57 respectively, were used as constants in the theoretically derived eqn (10), with K_1 =1 t ha^{-1} to give

$$R_w = 6.26/(1+W) + 5.57 \qquad (15)$$

This calibrated equation was further verified by considering the N:P ratio in different crops at different stages of growth using experimental data in datasets B and C.

4. The validity of the model and equation (15) is supported by agricultural and ecological experiments

Agricultural experiments (dataset B) that are entirely independent of those used for developing the model give the relationship between N:P ratio and W that is similar to calculated from eqn (15) (Fig. 2). The dataset B includes measurements made throughout the growth period on the C4 crop maize and on five C3 crops. The values of W range from 0.1 to 27 Mg ha^{-1} and yet are in reasonably good agreement with eqn (15).

As N:P ratios are particularly sensitive to changes in W, when W is small, we tested the validity of the model against measurements made in the early stages of crop growth. In dataset A, the average N:P ratio of crop seedlings, for values of W < 0.2 Mg ha^{-1}, is 9.8 compared with 11.16 obtained by substituting W=0.1 Mg ha^{-1}in eqn (15). The average ratio of seedlings in dataset B with W < 2 Mg ha^{-1} was 9.3, which is close to the predicted value of 8.65 obtained by substituting W= 1 Mg ha^{-1}in eqn (15). **[Tables S3 and S4 in Supplementary Information].**

According to the model, the average N:P ratio of the growth related tissue, R_g, is an important plant characteristics that does not vary much during growth (Fig.1, Table 1). As a result changes in N:P ratio during the growth is mainly driven by changes in the biomass of the growth related tissue, but not by its N:P ratio. Because leaves consist mainly of growth related tissue the average values of N:P ratio in leaves should be close to the R_g found in the study. We have studied ecological measurements on leaves to check this proposition, namely, to confirm

that leaf %P is proportional to leaf %N with a proportionality constant of 1/11.83= 0.0843. Fig 3a gives leaf %P plotted against leaf %N on logarithmic axes for the 206 sets of data on the leaves of herbage species (Kerkhoff *et al*., 2006) and Fig. 3b gives a similar plot for 177 sets of data on the leaves of evergreen trees of deciduous shrubs (Wright *et al*., 2004). The lines are calculated by assuming the N:P ratio is always equal to that of the growth related tissue namely, 0.0843 and appear to give a good representation of the average values of the data. Leaf photosynthesis usually ceases when the leaf nitrogen concentrations are low (Nátr, 1975) so such leaves could not be regarded as growth related tissue. For this reason we have confined formal statistical analyses on leaf measurements to leaves with a %N greater than one. The details of these analyses are given in **Table S5 in supplementary information.** RMA regressions of $\log_{10}$%P against $\log_{10}$%N were carried out separately on the Kerkhoff and the Wright data. The means sums of squares of the deviations between measured and calculated values (from the regressions) of $\log_{10}$%P and vice versa were determined. They were also determined when the values were calculated from P/N = 0.0843. Although the gradients of the RMA fits were greater than one indicating a curvature in the relationships, the degree of agreement between measured and calculated values was not significantly different whether calculations were from the RMA fitted equations or from P/N = 0.0843. The ratio of the mean sum of squares of deviations for calculations with the latter equation to that with the former equations was 1.14 which is not significant at p = 0.05. Thus P/N = 0.0843 gives a good description of the data. This is further supported by the finding that the average P:N ratios of the Kerkhoff and of the Wright leaves were not significantly different from 0.0843. Therefore the P:N ratio of wild leaves having a %N greater than one is similar to that inferred from our model (eqn 10) and experiments on field vegetables.

5. The calibrated theoretical relationship in eqn (15) predicts optimal N and P concentrations in the dry biomass of different crops

Relatively few experiments (given in Fig.2) have been published where sequential measurements were made of W, %N and %P. on crops grown with near optimum levels of nutrients. However, a considerable amount of other data exists on the optimal %N and %P that does not include W for different crops and conditions. We therefore sought to find how far they could be predicted with eqn (15). The methods were as previously described under dataset C. The data includes N:P ratios (dataset C) at each of four growth stages of four different C3 cereals, grown with 'adequate nutrition', that were derived from Tcierling's (1978) review of nationwide Russian experiments. Comparisons between measured N:P ratios and those calculated with eqn (15) are given in Fig.4. An upper limit for the s.e of the measurements is 0.7 (see legend to Fig.4) indicating that the least significant difference is about 1.4. As in 12 out of the 15 comparisons the difference between measurement and calculated N:P ratio is less than 1.4, it follows that agreement between measurement and calculation is reasonably good. Bergman (1992) in his review of world-wide literature gives the ranges of %N and %P of immature tissues of numerous species required for good growth. Altogether the data set includes 124 records (dataset C), the average of the range of %P of each record was plotted against the average %N. They are linearly related and are plotted in Fig. 5 which also gives the linear relationship predicted with eqn (15). The slopes of the two relationships are almost identical but the intercept of the predicted relationship is about 0.1% higher that that for the measurements. Measurements of %P and %N of the above ground biomass made at intervals during the growth of tall fescue and rye grass grown with near optimum conditions (Salette, 1990) are also in a good agreement with that obtained with eqn (15) (Fig. 6).

DISCUSSION

Our study indicates that the N:P ratios in the growth related tissues of many crops are about 11.83, and are similar to those of the leaves of many naturally grown species (Fig.3). This value is in the range of that found for micro-organisms (Sterner and Elser, 2002) and is close to the averages of 12.65 and 13.64 for terrestrial and fresh water autotrophs (Elser *et al*., 2000a). These similarities imply that the same cellular processes, such as changes in the transcribed rRNAs (Elser *et al*., 2000b) and in RNA:protein ratios (Karpinets *et al*., 2006), may underlie the relationships between the N:P ratios of growing cells and their growth rate whether in plants or micro-organisms. The nutrient requirements of this conserved molecular machinery could dominate the N and P requirements for cellular growth and thus explain their close balance and synergy in natural ecosystems (Elser *et al*., 2007).

An alternative to the proposed mechanistic modelling of the crop N:P ratio is to fit an empirical model to the experimental data. One widely used model in crop nutrition (Lemaire, 1997) considers the relative changes in %N and %P during growth of the whole crop in terms of simple scaling relationships with W; these are $\%N = aW^b$; $\%P = cW^d$ and $N:P = (a/c)W^{(b-d)}$ where a, b, c and d are coefficients that are fitted separately for each experiment. The model gave good fits to each of 5 sets of our test data **[Table S6 in supplementary information];** an example of which is given in Fig.7. As both the coefficients b and d were always negative and b was less than d the relative decline in %N was always greater than that of %P. The model also indicates that N: P will never remain constant unless b = d. Our mechanistic model also gave a good description of the dependence of N:P on W. In addition, the calibrating coefficients have a clear biological sense representing the N:P ratios in each of the main plant tissues. One of these

parameters appears to be constant, as mentioned above, not only across plants, but also across microbial organisms which demonstrates that proposed mechanistic model quantifies global regularity in biological plant species.

In the Introduction we emphasised that in accord with the Growth Rate Hypothesis the specific growth rate of heterotrophic organisms increases with decrease in N:P ratio (Elser *et al*., 1996; Elser *et al*., 2000b; Sterner and Elser, 2002). It was also pointed out that a similar relationship had been found for leaves (Niklas, 2006). In fact we found limited support for the relationship as the N:P ratio of the growth related tissue, which is thought to consist mainly of leaves, for the experiments in data set A was negatively, although weakly, correlated with the growth rate K_2. Nevertheless in our studies the N:P ratio of the whole plant declines as plants grew and their specific growth rate fell, which is in accord with Ågren's predictions (Ågren, 2004) for whole plants with SGR's (<0.1 day^{-1}) that are similar to the SGR's of our data. According to our model this difference occurs because whole plants, unlike unicellular organisms and individual parts of plants, consist of different tissues, each with different functions and thus different demands for N and P. During growth the proportions of the tissues change leading to changes in the total requirement of the whole plant for N relative to that of P. Our model is a simplification of the various processes but its validity is supported by much experimental information covering widely different species grown in different ways. It illustrates how strongly changes in the relative proportions of the growth and storage related tissues dominate the N:P ratio of the whole crop. For example, according to eqn (4), the weight of growth related tissue as a fraction of total plant weight, W, is equal to $1/(1+W)$ which indicates that the fraction declines sharply with small increases in W; in fact the fraction drops from 1 to 0.5 with an increase in W from 0 to 1 Mg ha^{-1}. As the average N:P ratio of this tissue 11.83 and that of storage related tissue is 5.58 it follows that there is a very sharp fall in the N:P

ratio of the whole plant with small increases in W as found experimentally (Fig. 2). It thus supports the view that N:P ratio of crop biomass grown with optimum concentrations of nutrient is greatly affected by the partition of assimilates to different tissues during growth.

The low N:P ratio of field vegetables (R_w) compared with that of much standing mass of natural communities deserves comment. The low N:P ratio is dominated by the low N:P ratio of the storage related tissues (R_s) which is subsequently used for reproduction and thus requires a high %P. As the average weight of the standing biomass of many natural communities is much greater than that of crops most of it could have consisted of wood which contains only very small amounts of nutrients (Altman and Dittmer,1964). In consequence the N:P ratio could be largely determined by that of the growth related tissue and be approximately 11.83 irrespective of the total weight of biomass. Indeed, a survey of such communities indicated that they had an N:P ratio of approximately 13 irrespective of the total weight of biomass (Kerkhoff et al., 2005).

Ecological studies emphasise the similarities in the relationships between %N and %P in the dry matter of leaves grown on soils of differing fertility (Niklas *et al*., 2005), whereas crop studies emphasise the dependence of crop %N and of %P on soil nutrient levels (Lemaire, 1997; Bollons and Barraclough, 1999). In this paper we show that the average N:P ratio of naturally grown leaves in a large dataset is on average similar to the N:P ratio of the growth related tissue of crops grown with near optimum levels of nutrients. As growth related tissue is thought to consist mainly of leaves, the average N:P ratio of natural leaves appears to be that expected with optimal plant nutrition. Presumably the leaves of natural vegetation have adapted over long periods to the stable but low soil nutrient levels, by various feed back mechanisms in such a way that the different nutrients limit growth simultaneously (Bloom *et al*., 1985; Gleeson and Tilman, 1992; Knecht and Göransson, 2004) and that when this occurs the N:P ratios are similar to those obtained for plants grown with the optimum fertilizer levels. Arable crops are in the ground for

much shorter periods, have much less time to adapt to the nutrient regimes, so homeostasis is less marked and plant nutrient concentrations are more dependent on soil nutrient levels. They have also been bred for rapid growth and high yields, possibly at the expense of their root systems and their ability to withstand soil nutrient stresses. Thus inter-site variation in growth of crops could be much more dependent on soil nutrient levels than that of natural vegetation. Also it is possible that the N:P ratio of the growth tissue is approximately the same over a wide range of natural and crop species and the soil nutrient affects on plants are entirely accommodated by differences in the N:P ratios of the storage related tissues.

Our model could improve crop nutrition by providing general diagnostic criteria for nutrient imbalance and by its incorporation into dynamic models for crop response to fertilizers, as described in the Introduction. Nevertheless some characteristics of the experimental data used for developing eqn (15) impose limits on its application. It may not apply when W is greater than 20 Mg ha^{-1} as most values of W in the experiments were less than this. It may not apply after the onset of appreciable leaf senescence as the field vegetables were harvested before the onset of senescence. An obvious major uncertainty in these studies is the extent to which deviations from optimum applications of N and P fertilizers have affected the measured N:P ratios. Less obvious is the error that may have resulted from setting K_1 = 1 Mg ha^{-1} for all crops. It is the value of W when growth rate is half the maximum. Such a low value seems to be appropriate for most of the field vegetables grown in the field experiments as these were grown at high plant densities, and complete crop cover and maximum growth rates were attained whilst W was small. Setting K_1 = 1 Mg ha^{-1} for all crops was based on the excellent fits that were obtained with this value of K_1 in fitting eqn (11) (Table 1). However, K_1 and K_2 are highly correlated in such fits and equally good fits can be obtained with different combinations of values of each of these coefficients and it may be that K_1 should be greater than 1 for widely

spaced crops. The same uncertainty exists for eqn (15) governing the dependence of N:P ratio on W. Increasing K_1 in eqn (10) and also in eqn (15) results in the N:P ratio declining less rapidly with increase in W. This could explain why R_w of Brussels sprouts declined less with increase in W as it was grown at a far wider spacing than the other crops. Wide plant spacing depressed the decline of %N of lucerne with increase in plant mass compared with close spacing (Lemaire and Gastal, 1997) which could be explained in terms of the interception of light (Hirose and Werger, 1987; Lynch and Gonzalez, 1993). Spacing might therefore be expected to affect the dependence of N:P ratio on W.

We conclude that the same physiologically derived equation with two constants gives a good prediction of the changes in N:P ratio in the biomass of a wide range of crops grown, in different ways, with near optimal nutrition. The model also gave good estimates of the relation between %P and %N in the leaves of natural vegetation provided the leaf %N was greater than one. The N:P ratio of crop biomass declines sharply with increase in plant mass per unit area because of an increase in the proportion of storage related tissue which has a low N:P ratio relative to growth related tissue which has a high N:P ratio.

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APPENDIX

Table A1

Supplementary Information

Table S1 Effect of variation in the ratio C_{Ps}/C_{Pg} on R_w

Table S2 Details of datasets A, B and C with references to the sources of data

Table S3 N:P ratios for values of $W < 0.2$ Mg ha^{-1} from dataset A

Table S4 N:P ratios of $W < 2$ Mg ha^{-1} from dataset B with references to the sources of data

Table S5 Statistical analyses of predictions of P:N ratios of wild leaves

Table S6 Empirical model fits measurements of %N and %P during crop growth

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Legends to figures

Fig. 1. Relationships between %N/%P and *1/(1+W)* with W in Mg ha^{-1} for each experiment from database A; the lines fitted by the RMA procedure. The data are for single experiments, namely French beans 73, pea 72, onions 92, swede 72, and turnip 72 and the corresponding values of r^2 are 0.85, 0.90, 0.80, 0.91, 0.73 respectively. Each of the three different symbols for the onion data refers to a different variety.

Fig. 2. Literature measurements (database B) of the ratio, %N/%P and W in Mg ha^{-1}during growth of crops grown with near optimum levels of nutrients and predicted dependence of the ratio on W by eqn (15). Details and sources of data are in **[Table S2 in Supplementary Information].**

Fig. 3. Comparison between predicted relationships (lines) calculated for the growth related tissue (%P = %N/11.83) and measurements (symbols) of the leaves of wild plants obtained in surveys.). **A** gives all 206 measurements for a survey of the leaves of herbage wild species referred to in Kerkhoff *et al*. (2006) and kindly provided by Professor Kerkhoff. **B.** gives the 147 leaf measurements with %N > 0.5 % on evergreen trees (open circles) and 30 measurements on deciduous shrubs (closed circles) reported in Wright *et al*. (2004).

Fig. 4. Estimated (closed columns) and predicted values of %N/%P (hashed columns) at tillering ear (Stage of growth 1), ear emergence (Stage of growth 2), booting (Stage of growth 3) and flowering (Stage of growth 4) of C3 cereals grown with optimum nutrients in Russia. An upper limit for the average s.e. for each of the closed columns is 0.702 (d.f. =10). It was calculated from the high order interactions in an analysis of variance for this data when combined with associated data for the same crops grown with excess nutrients (Tcierling, 1978).

Fig. 5. The predicted relationship (continuous line) between optimum %P and %N (%P = 0.1152×%N + 0.0788 r^2 = 0.99) as described in Dataset C of the Dataset section and 124 advisory estimates (symbols) from the literature for leaves and whole plants of many species (Bergman, 1992). RMA regression (dashed line) for these advisory relationships gives %P = 0. 1208 × %N -0.03436, r^2 = 0.61; the s.e. of the gradient is 0.0068.

Fig. 6. Predicted relationship (continuous line) between optimum %P and %N (%P = 0.0981 %N + 0.1151, r^2 = 0.97and measured values (calculated from authors regressions which had r^2 >0.89) for grasses grown with near optimum levels of nutrients in France (Salette, 1990)

Fig.7. The calculated relationship between N:P and W (continuous curve) and the measured values of N:P for the turnip 72 experiment. Calculations were made with N:P =$(a/c)W^{(b-d)}$ in which the coefficients a and b were obtained by fitting %N = aW^b and c and d by fitting %P = cW^d . The fitted values of a and b were 4.375 and -0.157 (r2=0.742) and of c and d were 0.614 and -0.0488 (r2=0.644).